AACC Guidance Document on Laboratory Testing for the Assessment of Preterm Delivery

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INTRODUCTION

Spontaneous preterm birth (PTB) is a delivery of an infant prior to 37 weeks gestation as a result of preterm labor, preterm premature rupture of membranes (PPROM), and/or cervical insufficiency. Prematurity and its complications are primary contributors to perinatal mortality and morbidity, and it is the second most common cause of infant death in the United States (1). Additionally, premature birth places surviving infants at greater risk of low birth weight, respiratory distress syndrome, underdeveloped organs, neurodevelopmental disabilities, cognitive impairment, visual and hearing impairments, developmental coordination disorders, and behavioral and emotional difficulties (2). In addition to decreased quality of life and emotional stress caused by these long-term health complications, they also impose a significant financial burden. A 2005 Institute of Medicine report estimated the overall cost of PTB in the United States at $26 billion annually and this number remained essentially unchanged in 2018 (3, 4). This figure included the cost of early intervention and delivery, direct medical care of preterm infants up to 5 years of age, as well as lifetime medical/special education costs and lost productivity for those with specific developmental disabilities associated with PTB.

The premature birth rate in the US rose to 10.0% in 2018, which was a modest increase relative to the 9.9% rate observed in 2017 (4). The rate of PTB had been declining until 2014 (9.6%), but has risen steadily since that time, despite efforts by the March of Dimes, the Healthy People 2020 initiative, and other prominent organizations. These programs aimed at reducing the rate of PTB have been hindered by a limited understanding of the underlying mechanisms, a lack of therapeutic options that directly target these pathways, and an inability to identify which women will benefit from therapeutic intervention. The majority of the increased PTB rate has been due to late PTBs, 34/7 until 36/7 weeks, while the rate of early PTB (less than 34 weeks) has remained essentially unchanged (5). This may be due in part to an increase in multi-fetal pregnancies from assisted reproductive technologies, a known risk factor for PTB. However, the number of singleton pregnancies resulting in preterm birth has also increased in recent years, indicating other causes for the increased rate of PTB, including increasing maternal age (5, 6). Furthermore, there is a noticeable racial disparity in the prevalence of PTB in the USA, as approximately 14.1% of black infants, 11.3% of American Indian/Alaska Native, 9.7% of Hispanic, and 9.1% of white infants are born preterm (5). Importantly, these differences cannot be fully explained by differences in socioeconomic status or maternal behavior and several studies have implicated certain genetic variants as risk factors for PTB (7, 8). While the precise mechanisms leading to normal parturition are not fully elucidated, there are several proposed etiologies leading to premature delivery, including maternal or fetal stress, inflammation, decidual hemorrhage, and/or pathological uterine distension (9).

Identification of women who will deliver preterm is critical to allow selective initiation of appropriate therapy, while preventing unnecessary treatment of women who will deliver at term. Administration of antenatal corticosteroids to accelerate fetal lung maturation improves outcomes among infants who are born preterm, while antenatal magnesium sulfate is often administered to improve neurologic outcome, and tocolytics are used to prolong the time to delivery when clinically appropriate (10-12). However, identifying individuals at risk of delivering prematurely is inherently challenging, even in those with symptoms of preterm labor. The single best predictor is a personal history of previous preterm delivery, which increases the risk of a subsequent preterm delivery by 2.5-fold (13). A number of other risk factors related to maternal age, social behavior, medical history, and socioeconomic status have been described, but none of them reliably identifies individual women who will give birth prematurely (14). One of the challenges of assessing risk for PTB is the ubiquitous nature of signs associated with the onset of labor, including uterine contractions, pelvic pressure, backache, or increased vaginal discharge. These signs are nonspecific and are common among pregnant women who do not deliver.
prematurely. Even in the presence of cervical change with uterine contractions, correctly identifying women in preterm labor who will ultimately deliver prematurely is difficult. Thirty percent of women in preterm labor (PTL) will have spontaneous resolution, and 50% of women who have been hospitalized for PTL will go on to deliver at term (15).

Given the nonspecific nature of clinical symptoms, there has been considerable interest in identifying biomarkers to predict which women will deliver preterm. Since PTB has multiple etiologies, a number of different biomarkers have been investigated in women with signs and symptoms of preterm birth. While numerous studies have addressed the utility of these biomarkers, none has demonstrated definitive clinical value. As a result, there is considerable variation in the incorporation of these biomarkers into clinical practice, as well as a lack of consensus among professional societies regarding their utility (16, 17). This guidance document describes the currently available biomarkers of PTB, summarizes the literature evaluating their diagnostic performance characteristics, and provides recommendations for their use in clinical practice.

WHAT IS THE PRE-TEST PROBABILITY OF PRETERM BIRTH IN THE UNITED STATES AND WHAT ASSAY PERFORMANCE CHARACTERISTICS ARE REQUIRED TO DEMONSTRATE CLINICAL BENEFIT?

In the U.S., the percentage of symptomatic women who deliver within 7 days is consistently less than 5% (18-20). As such, the pre-test probability that a symptomatic woman will not deliver within 7 days is >95% and a test with 50% sensitivity and 50% specificity (equivalent to a coin flip) would provide a negative predictive value (NPV) >95%. In conditions with low prevalence, a high positive predictive value (PPV) and positive likelihood ratio (LR+) are most clinically useful since a positive test result substantially increases the pre-test probability of disease and separates the small number of affected patients from unaffected patients with similar symptoms (21, 22). Conversely, a high NPV provides limited clinical value in this patient population as it does not significantly reduce the pre-test probability of disease. In general, clinical laboratory tests are useful if they demonstrate a LR+ greater than 10 or a negative likelihood ratio (LR-) less than 0.1 (21, 23). LRs that do not reach these thresholds only modestly change the pre-test probability of disease.

• In populations with a pre-test probability of <5%, biomarkers should demonstrate a high PPV and high LR+ to provide clinical utility.
• A high NPV and low LR- does not substantially reduce the pre-test probability of PTB.

WHAT IS FETAL FIBRONECTIN (FFN)?

Fetal fibronectin (fFN), an embryonically produced form of fibronectin, is the most well-characterized of the available biomarkers for predicting preterm birth (PTB). fFN is a 440 kDa extracellular membrane glycoprotein that is thought to mediate implantation and placental-uterine attachment for the duration of pregnancy (24, 25). Using an antibody that targets an epitope unique to fFN, its presence has been demonstrated in amniotic fluid, placental tissue, and cervicovaginal secretions (25-27). Cervicovaginal fluid (CVF) fFN concentrations are highest in the first trimester of pregnancy before falling to undetectable concentrations in the second and third trimester, and ultimately increasing at term following fusion of maternal membranes around 22 weeks gestation (27). fFN may be measured using commercially available assays in either qualitative or semiquantitative lateral flow assay formats (26, 28). The qualitative device is FDA cleared for use in CVF samples from women who present between 24\(^{6/7}\) and 34\(^{6/7}\) weeks gestation with signs and symptoms of pre-term labor, intact amniotic membranes, and cervical dilation <3 cm. It is also approved for asymptomatic patients between 22\(^{6/7}\) and 30\(^{6/7}\) weeks gestation with a history of previous preterm delivery before 35 weeks 6 days. The semiquantitative device is not available for use in the U.S.

WHAT ARE THE PERFORMANCE CHARACTERISTICS OF FFN IN WOMEN WITH SYMPTOMS OF PRETERM LABOR?

In the first published study evaluating the diagnostic performance of CVF fFN, concentrations above 50ng/mL identified women who would deliver preterm with a sensitivity of 81.7% and a specificity of 82.5% (27). Since this landmark publication, numerous studies have assessed the clinical utility of fFN as a biomarker for predicting PTB and have demonstrated largely consistent results. Among patients with symptoms of PTL, a single fFN result (<50ng/mL) was associated with a NPV of ~99% and a PPV ranging from 6%-40% when used to predict preterm delivery within 7 days (29-32). Iams et al. demonstrated in a prospective cohort of 418 women presenting with symptoms of PTL that fFN concentrations >50 ng/ mL were 93% sensitive and 82% specific for predicting delivery within 7 days in women with cervical dilation <3 cm with a PPV of 29% and an NPV of 99% (29). Similarly, Peaceman et al. demonstrated an NPV of 99.7%, 99.5%, and 86.6% for delivery of an infant within 7 days, 14 days, and <37 weeks’ gestation, respectively, in symptomatic women with singleton pregnancies (30). However, the positive predictive value (PPV) did not exceed 20% at any of the time points tested.

The finding that fFN concentrations <50ng/mL could rule out delivery within 7 days in symptomatic women with a NPV exceeding 90% has since been replicated in multiple studies (33-35) and has led several to argue that the high NPV supports the use of fFN to rule out PTB within 7 days in symptomatic women. However, as the prevalence of PTB within 7 days in symptomatic women is consistently less than 5%, any test with
50% sensitivity and 50% specificity will demonstrate a NPV of >95%. Consequently, the high NPV documented in these studies does not indicate strong diagnostic performance but rather reflects the low prevalence of PTB in the study population.

Confirming the limited clinical utility in this patient population, a meta-analysis of cohort studies evaluating the diagnostic accuracy of CVF fFN in predicting PTB within 7 days in 5355 symptomatic patients with a median pre-test probability of 8% found an overall LR+ of 4.20 and LR- of 0.29 (23). A second similar meta-analysis of symptomatic women with a median pre-test probability of 3% found an overall LR+ of 5.42 and LR- of 0.25 (36). In both meta-analyses, neither the LR+ nor the LR- met the threshold required (>10 and <0.1, respectively) to significantly change the pre-test probability.

- In populations with a pre-test probability of 3%, a positive fFN result modestly increases the post-test probability to ~15%, while a negative result does not substantially reduce the post-test probability.
- fFN does not provide sufficient assurance to either rule in or rule out PTB within 7 days.

**DOES FFN IMPACT CLINICAL OUTCOMES IN SYMPTOMATIC WOMEN?**

The high NPV of fFN for ruling out PTB within 7 days has led several groups to argue that its utility lies in adjudicating symptomatic women with a cervical dilation < 3 cm and an equivocal cervical length as measured by transvaginal ultrasound (between 20-30 mm) (37). This approach is hypothesized to identify women who are unlikely to deliver within 7 days, reducing unnecessary treatments and healthcare expenditures. However, randomized controlled trials that assess patient outcomes and healthcare utilization by implementing fFN testing are lacking and existing studies have found conflicting results (15).

In support of fFN testing, one study screened women asymptomatic for PTL by cervical length and fFN and found a reduction in the incidence in PTB relative to the women whose physicians did not have access to cervical length and fFN results (38). Another retrospective study attempting to implement standardized protocols for fFN testing and interpretation of these results predicted a reduction in hospital admissions by 56% (39). One study comparing preterm labor admissions over 2 separate 12-month periods (with and without fFN) found fFN testing significantly reduced admissions (28.1% without fFN vs 17.0% with fFN) and saved approximately $486,000 without affecting the frequency of preterm delivery, admissions to the NICU, or NICU length of stay (40). In contrast, a randomized trial of 100 women found that access to fFN results provided no change in physician behavior or health care costs (41). A systematic review of 6 trials including 546 women concluded that patient management based on the knowledge of fFN did modestly reduce the relative risk (RR) of PTB before 37 weeks (RR 0.72).

However, there was no effect on PTB before 34 weeks (RR 1.09) or frequency of maternal hospitalization (RR 1.06) (42). A separate study assessing implementation of fFN on physician decision-making concluded that a greater emphasis was placed on positive results, rather than negative, which ultimately led to increased utilization of health care resources (43). There is limited and conflicting literature on the impact of fFN testing on reducing PTB risk, influencing physician decisions, and reducing health care costs. Randomized controlled trials are required to formally demonstrate the utility (if any) of fFN testing.

- fFN testing does not consistently improve clinical outcomes, as measured by reduced hospitalization rates, rates of PTB, or health care costs.

**WHAT ARE THE PERFORMANCE CHARACTERISTICS OF FFN IN ASYMPTOMATIC WOMEN?**

Studies assessing the use of fFN for predicting PTB in asymptomatic women have demonstrated a relative lack of clinical utility. Goldberg et al. performed routine fFN screening on 2929 asymptomatic pregnant women every 2 weeks from gestational weeks 22-30. Using a single cutoff of 50 ng/mL, fFN measured between 22-24 weeks gestation was able to detect PTB at 28 weeks with a sensitivity of 63% and a specificity of 98% (44). However, sensitivity decreased considerably when the definition of PTB was extended to include later gestational ages and when fFN testing was performed after 24 weeks. The largest study to date assessed 9410 nulliparous women with singleton pregnancies for both vaginal fFN concentrations and transvaginal cervical length (45). Using a cutoff of 50 ng/mL, fFN concentrations measured at 23-30 weeks were able to predict PTB with a PPV of 10.7%, a NPV of 95.7%, a sensitivity of 8.1%, and a specificity of 96.8% (45).

- fFN should not be measured in asymptomatic women due to its low sensitivity and PPV.

**DO TIERED CUTOFFS IMPROVE DIAGNOSTIC PERFORMANCE?**

In a study of 300 symptomatic women between 220/7 and 356/7 weeks gestation, with a spontaneous PTB rate of 5.7% within 14 days of testing, the LR+ were 2.02, 4.04, 9.69, and 14.12, and LR- were 0.30, 0.29, 0.44, and 0.66 at cutoffs of 10, 50, 200, and 500 ng/mL, respectively (46).

- While promising, further studies are required to demonstrate improved clinical outcomes associated with semi-quantitative fFN testing.

**ARE THERE ANY TRENDS IN THE NUMBER OF LABORATORIES PERFORMING FFN TESTING?**

Recent College of American Pathologists (CAP) proficiency testing surveys (survey FF) have demonstrated modestly, yet consistently, decreased participation over the last 4 years, which
may reflect laboratorian and clinician recognition that fFN testing does not improve outcomes (Table 1).

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**WHAT IS IL-6?**

Interleukin 6 (IL-6) is a pleiotropic cytokine with well-characterized roles in the inflammatory response. Inflammation may occur in response to tissue damage, but it is also associated with physiological processes in the female reproductive system, including ovarian follicle rupture, blastocyst implantation, shedding of the endometrial lining during menstruation, and parturition (47, 48). As intrauterine inflammation is the most common cause of PTB and spontaneous delivery at term, IL-6 has been investigated as a possible biomarker for the evaluation of women with signs and symptoms of PTL (49, 50). Initial studies measured IL-6 in amniotic fluid and proposed its use as a surrogate marker of intrauterine infection. When measured via hepatocyte-stimulating factor assay and SDS-PAGE, amniotic fluid IL-6 concentrations were higher in women with preterm labor and intra-amniotic infection (median 375 ng/mL, range 305000) than in women with preterm labor without intra-amniotic infection (median 1.5 ng/mL, range 0-500). While the median values were different, there was substantial overlap in amniotic fluid IL-6 concentrations between the 2 groups. Median amniotic fluid IL-6 concentrations were also low in control groups consisting of mid-trimester women (median 10 ng/mL, range 2-27), at term but not in labor (median 13 ng/mL, range 0-60) and at term and in spontaneous labor (median 19.5 ng/mL, range 4-500) (51). A point-of-care test for the quantitative detection of IL-6 in amniotic fluid demonstrated a PPV of 21.8% and a NPV of 100% when used to detect microbial-associated intra-amniotic inflammation. A PPV of 80% and NPV of 84.1% were observed when used to predict spontaneous delivery within 7 days of amniocentesis (52, 53). When measured in second-trimester amniotic fluid samples, mean IL-6 concentrations were higher in women who experienced spontaneous delivery before 34 weeks (1.6 ng/mL ± 3.2) than those who delivered spontaneously at 37 weeks or later (0.8 ng/mL ± 1.2 ng/mL), although significant overlap was observed between the 2 groups. Amniotic fluid IL-6 concentrations were also negatively correlated with gestational age at delivery (54).

**WHAT ARE THE PERFORMANCE CHARACTERISTICS OF IL-6 IN THE PREDICTION OF PTB?**

As amniocentesis is not routinely performed in the evaluation of preterm labor, subsequent studies addressed the diagnostic performance of CVF IL-6 in the prediction of PTB. Woodworth et al. analyzed 660 remnant physician-ordered specimens collected for fFN testing in women with gestational ages ranging from 24 to 35 weeks (18). Of the 552 women from whom specimens were collected, 491 exhibited signs of PTL at the time of specimen collection, while the remaining 61 did not. Using a cutoff of 250 ng/L, CVF IL-6 had a PPV of 12%, NPV of 99%, LR+ of 6.1, and LR- of 0.47 for predicting delivery within 7 days of specimen collection. Results were similar for delivery within 14 days, with PPV and NPV of 16% and 97%, respectively. In this study population, prevalence of PTB was 2.1% (14/660) and 4.7% (31/660) within 7 and 14 days, respectively. Other studies summarized in Table 2 confirmed the high NPV and low PPV, which increased with increasing pre-test probability.

- In populations with a pre-test probability of 2%, a positive IL-6 result modestly increases the post-test probability to ~15% while a negative result does not substantially reduce the post-test probability.
- IL-6 does not provide sufficient assurance to either rule in or rule out PTB within 7 days.

**HOW DOES IL-6 COMPARE TO FFN?**

In a head-to-head comparison using the same sample set, CVF fFN and IL-6 measurements demonstrated nearly identical performance characteristics (18). To predict delivery within 7 days, fFN was associated with a PPV and NPV of 13% and 100%, respectively while IL-6 was associated with a PPV and NPV of 12% and 99%, respectively. When stratified by ethnicity, IL-6 performed modestly better in African-American women (PPV 15%, NPV 99%) relative to Caucasian women (PPV 7%, NPV 99%) a result which correlated with an increased prevalence of chorioamnionitis and bacterial vaginosis in the African-American subset of patients (60). Other studies have supported this finding and have observed that PTB in African-American women is primarily associated with uterine inflammation while precipitating factors in Caucasian women are more variable (61).

- IL-6 and fFN demonstrate similar diagnostic performance characteristics.
- IL-6 shows modestly improved performance in African-Americans but this is not sufficient to significantly impact clinical decision making.
WHAT IS PAMG-1?

Placental alpha microglobulin-1 (PAMG-1) is a glycoprotein secreted from decidual cells that exhibits heme and radical binding modalities, as well as reductase activity (62, 63). Collectively, these features could allow AMG-1 proteins to mitigate damage from oxidative stress and initiate tissue repair mechanisms, indicating that PAMG-1 may serve a protective role in pregnancy. Although PAMG-1 is detectible in amniotic fluid, maternal blood, and CVF, the relative concentration of PAMG-1 in amniotic fluid (2000-25 000 ng/mL) is significantly higher than in maternal blood (5-25ng/mL) and CVF with intact membranes (0.05-2 ng/mL) (64). Immunochromatographic assays have exploited this difference in concentration to detect premature rupture of membranes (PROM) with reported clinical sensitivity of 99% and specificity ranging from 88%-100% (64). PAMG-1 can be measured in a qualitative, lateral flow format that is FDA-approved for use between 240/7 and 346/7 in assessing the risk of spontaneous PTB in symptomatic women with singleton gestations and cervical dilation <3 cm.

WHAT ARE THE PERFORMANCE CHARACTERISTICS OF PAMG-1 IN THE PREDICTION OF PTB IN POPULATIONS WITH RATES OF PTB <5% AND HOW DOES IT COMPARE TO FFN?

Studies evaluating the performance of PAMG-1 as a diagnostic tool in the prediction of PTB have consistently demonstrated 3- to 4-fold higher PPVs relative to fFN, although the absolute PPVs are variable and highly dependent on prevalence (19, 20, 65-67). While the 2 biomarkers exhibit equivalent utility in ruling out spontaneous PTB within 7 days, studies indicate that PAMG-1 may be diagnostically superior for predicting spontaneous preterm delivery in symptomatic women.

Two studies comparing fFN and PAMG-1 demonstrated fewer false positive PAMG-1 results and overall improved diagnostic performance of PAMG-1 relative to fFN. One retrospective cohort study evaluated the 2 biomarkers during 2 separate 1-year periods (fFN used exclusively one year, PAMG-1 used exclusively the second year) in women with a singleton pregnancy presenting with symptoms of preterm labor between 24 and 35 weeks (20). The prevalence of spontaneous preterm delivery within 7 days was 2.6% during the fFN year and 3.3% during the PAMG-1 year. Sensitivity, specificity, PPV, NPV, LR+, and LR- were 50%, 97%, 47% 86, 29, 79, and 12, 96, 47, 100, respectively, for PAMG-1 and 30%, 98, 94, 99, 3.2, and 0.8, respectively, for fFN. In a second, prospective study, both fFN and PAMG-1 were measured in women presenting with symptoms of preterm labor between 24 and 35 weeks gestation (19). The prevalence of spontaneous, preterm birth within 7 days in women with singleton gestations was 0.9%. Sensitivity, specificity, PPV, NPV, LR+, and LR- in this subset of patients was 50.0%, 98.4%, 23.1%, 99.5%, 31.3, and 0.4, respectively, for fFN.

- In populations with a pre-test probability of 5%, a positive PAMG-1 result increases the post-test probability to ~20-30% while a negative result leaves the post-test probability essentially unchanged.
- PAMG-1 does not provide sufficient assurance to either rule in or rule out PTB within 7 days.
- PAMG-1 demonstrates improved PPV and NPV relative to fFN, but the majority of women with a positive PAMG-1 result do not deliver within 7 days.
WHAT ARE THE PERFORMANCE CHARACTERISTICS OF PAMG-1 IN THE PREDICTION OF PTB WHEN INITIAL SCREENING INCREASES THE RATE OF PTB IN THE POPULATION UNDERGOING TESTING?

In patient populations with a higher prevalence of spontaneous preterm delivery within 7 days (17%-27%), PAMG-1 has a PPV of approximately 75%, which likely would provide clinical value as the majority of women with a positive test result would go on to deliver prematurely (66, 67). These studies incorporated routine use of transvaginal ultrasound to exclude lower risk women on the basis of cervical length, thereby increasing the prevalence of PTB in the study population. Cervical length assessment is not routinely performed in the U.S., and studies that follow a diagnostic approach that does not include a triage step to exclude low risk women consistently report a much lower prevalence (0.9%-3.3%) (19, 20).

- In populations with a pre-test probability of 20%-25%, a positive PAMG-1 result substantially increases the post-test probability to ~75%.
- In this patient population, a positive PAMG-1 result may help to identify women likely to deliver within 7 days.

IS THERE VALUE IN COMBINING MULTIPLE BIOMARKERS?

One limitation of the currently available test methods is that they measure a single protein, each of which is associated with a specific etiology of PTB (IL-6—intrauterine infection/inflammation, fFN—matrix breakdown, PAMG-1—presence of amniotic fluid). As PTB is a multifactorial condition, some have proposed the use of multimeter panels to more accurately predict women who will deliver prematurely. In a prospective cohort of 118 symptomatic women between 24 and 34 weeks gestation, preterm delivery risks following a fFN result $>$50 ng/mL or IL-6 $>$100 pg/mL were 5.48 and 1.57, respectively. Combining the 2 biomarkers did not improve diagnostic performance relative to fFN alone (57). In an observational cohort study of 286 asymptomatic women between 16 and 24 weeks, a panel of 7 novel biomarkers measured in CVF demonstrated a PPV of 27%, NPV of 100%, and LR+ of 3.88 for the prediction of birth <37 weeks (68). The ratio of insulin-like growth factor-binding protein 4 (IGF-BP4) to sex hormone-binding globulin (SHBG) in maternal serum samples collected between 19.5/7 and 21.5/7 weeks gestation has been proposed as a predictor of subsequent preterm delivery (69, 70). However, substantial overlap in predictor scores was observed between cases who delivered before 32 weeks and controls who delivered at or after 32 weeks. In patients with predictor scores in the upper quartile (associated with highest risk of preterm birth), PPV ranged from 2%-7% (70).

- While promising, multi-marker panels evaluated to date do not demonstrate improved diagnostic performance relative to single biomarkers.

DO OTHER PROFESSIONAL SOCIETIES RECOMMEND THE USE OF BIOMARKERS IN THE EVALUATION OF PRETERM LABOR?

The only biomarker currently recommended by American or European guidelines for predicting PTB in patients with PTL is fFN (16, 71, 72). However, there is disagreement among professional societies about its utility. In its 2016 practice bulletin on preterm labor, the American College of Obstetricians and Gynecologists (ACOG) does not recommend routine use of fFN to stratify risk for pre-term delivery (15). ACOG cites the low PPV of fFN and the lack of randomized controlled trials that demonstrate that a negative fFN result sufficiently changes physicians’ practices to reduce unnecessary use of resources (41). This position from ACOG is an update from their previous stance in 2003, when they stated that negative fFN results may be useful to avoid unnecessary interventions (73); likely owing to the findings of more recent randomized trials. In contrast to ACOG, the Society for Maternal-Fetal Medicine (SMFM), a US society comprised of mostly physicians and scientists, does endorse the use of fFN testing (16). Specifically, their 2016 statement on the use of biomarkers recommends that fFN be used in women presenting with symptoms of PTL prior to 34 weeks and a "borderline" transvaginal ultrasound demonstrating cervical length between 20-29 mm. The updated 2019 guidelines from the National Institute for Health and Care Excellence (NICE) in the UK also recommends the use of fFN in symptomatic women, but only if the measurement of cervical length is not available or not acceptable (19). No societies advocate for the use of tiered or higher cutoffs for fFN concentrations (72). Supplementary to the guidelines stated above, there is also a clear lack of consensus among individual experts as to the utility of fFN and when it should be used (74-76). As a result, some have called for standardization and protocolization of fFN testing, limiting its use only to women with symptoms of PTL and intermediate cervical length (24, 25). In contrast to dissenting opinions about the utility of fFN in women symptomatic of PTL, professional societies are unanimous in their recommendation against the use of fFN as a screening test in asymptomatic women.

To date, no professional societies have adopted recommendations for measuring IL-6 or PAMG-1 in the assessment of preterm labor and delivery.

- Laboratorians should discuss professional society guidelines and other available literature with their colleagues in Obstetrics and Gynecology to collectively determine an institutional testing strategy that best meets the needs of their patient population.

CONCLUSION

At this time, AACC does not recommend measurement of fFN, PAMG-1 or IL-6 in the routine evaluation of all women with symptoms consistent with preterm delivery. Identifying a
single biomarker for the prediction of women who will deliver preterm has been difficult because a number of different pathophysiological processes can lead to PTB. Racial/ethnic differences in the precipitating factor(s) responsible for PTB have made the development of a single biomarker that can identify all women who will deliver prematurely even more elusive. Multi-marker panels have been proposed to overcome this limitation, but their diagnostic performance characteristics are no better than those of individual markers alone. However, PTB remains the leading cause of neonatal mortality and places surviving infants at greater risk of long-term neurological impairment and other complications (17). It also represents a substantial financial burden for medical centers caring for premature infants. Currently available biomarkers for the prediction of PTB provide limited value when incorporated into diagnostic algorithms most frequently used in the United States. One possible solution is to identify novel diagnostic tools with improved PPV to predict the minority of symptomatic women who will deliver prematurely. An alternative solution is to limit biomarker testing to high-risk women, thereby increasing the pre-testprobability of the population being tested and improving the PPV of currently available test methods. Both solutions have the potential to improve outcomes but will require a more detailed understanding of the mechanisms responsible for initiating labor and delivery or the implementation of screening steps that limit testing to women at high risk of PTB.

Nonstandard Abbreviations
PPV, positive predictive value; NPV, negative predictive value; fFN, fetal fibronectin; IL-6, interleukin-6; PAMG-1, placental alpha macroglobulin-1; PTB, predicting preterm birth; PPROM, preterm premature rupture of membranes; PTL, preterm labor; LR+, positive likelihood ratio; CFV, cervicovaginal fluid; RR, relative risk; CAP, College of American Pathologists; IBP4, insulin-like growth factor-binding protein; SHBG, sex hormone-binding globulin; ACOG, American College of Obstetricians and Gynecologists; SMFM; Society for Maternal-Fetal Medicine: NICE, National Institute for Health and Care Excellence.

Author Contributions
All authors confirmed they have contributed to the Intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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